



Egyptian Journal of Plant
Protection Research Institute

www.ejppri.eg.net



**Evaluation of natural volatile oils for the management of greater wax moth adults
Galleria mellonella (Lepidoptera: Pyralidae) and their effect on activity of honeybee
colonies under apiary conditions**

Said, N. Ahmad; Naglaa, E. Ghazala and Dina, M. A. Taksira

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

ARTICLE INFO

Article History

Received: 17/10 /2022

Accepted: 28/12 /2022

Keywords

Honeybee, wax moth,
Galleria mellonella,
garlic, camphor and
menthol.

Abstract

This work was conducted in the apiary of Plant Protection Research Institute at Quantar, Qalubia to study the effect of three oils of plants (Garlic, *Allium sativum*), (Camphor, *Cinnamomum camphor*), (Menthol, *Mentha* sp.) and mixture of them on wax moth adults, *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) in honeybee colonies and on individual activity of honeybee colonies under field conditions through the period of 11/5/2020 to 27/7/2020. Results revealed that, garlic, camphor, and menthol oils were highly effective than mixture oils. The mean percentage mortality of adult wax moth after weekly sequence treatment could be arranged into the following descending orders as follows: 83%, 75%, 68% and 62% when using plates, garlic, camphor, menthol oils and mixture oils. The mortality had significant statistical differences at different oils at various post treatment intervals. All the essential oils were highly safe for adult honeybee workers.

Introduction

The honeybees infected by some pests such as the wax moth which is a highly destructive insect that attacks and destroy beeswax combs especially those in storage and can cause substantial losses to combs, hive materials all over the world (Shimanuki *et al.*, 1992 and Alemu *et al.*, 2014). Adult wax moths and larvae can also transfer pathogens of serious bee diseases, e.g. foulbrood. However, in colonies infested with this disease, feces of wax moth contain large amounts of spores of the causative bacteria, *Paenibacillus* larvae (Charrière and Imdorf, 1997).

During its development it builds silk-lined tunnels in the honey comb and feeds on honey, pollen, wax,

faces and destruction of honeycombs and subsequent deterioration of the weakened colonies. The efficacies of the tested materials were different. Thus, highly effective ones (Methyl salicylate, formic acid, clove and basil oils) were applied to protect wax combs stored ordinarily in the apiary (Ayman and Atef, 2007). The control adult of wax moth with certain volatile plant oils which seem to be safer and less contaminant to bees and humans. Such treatment methods with non - harmful products could contribute considerably to control this pest and reduce the risks of beehive product contamination and may give a possible solution for this apicultural problem.

Aromatic plants and their essential oils are very important sources

of many compounds that are used in different respects (Amer and Mehlhorn, 2006). Recent studies showed that many plant extracts have an effect on insects and mites (Ho *et al.*, 1994 and Shahera, 2007). The control LWM with certain volatile plant oils which seem to be safer and less contaminant to bees and humans (Abou El-Ela, 2014). All the concentrations of the aqueous neem seed extract more or less affect the population of the greater wax moth [*Galleria mellonella* L. (Lepidoptera: Pyralidae)]. The mortality increases with the increase in the concentration of the aqueous NSE (Izhar-ul-Haq *et al.*, 2008). The spraying aqueous solutions of 4 % of Egyptian propolis, Chinese propolis, cinnamon, clove and mint on the new combs of waxes will protect it from wax moth larvae infection since the tested extracts can reduce the life cycle of wax moth to 42.9 days at clove treatments comparing with 72.0 days at untreated combs, moreover, the tested extracts inhabiting egg hatchability recording only 29.1 hatch % at cinnamon treatment comparing with 86.3 % at untreated wax combs (Sanad and Mohanny, 2015). The oil of two plants were tested namely; Marjoram, *Origanum majorana* and Lemon Grass, *Cymbopogon proximus* were tested. To evaluate the relative efficacies of these materials as alternative control agent against GWM. Comparing the estimated LC10, LC50, LC90 values of present investigation of mentioned essential oils indicated that essential oils of *Origanum majorana* is more toxic to *G. mellonella* than the other essential oil of *Cymbopogon proximus* (Hussein *et al.*, 2014). The insecticidal activity of the essential oils peppermint *Mentha piperita* L., geranium; *Pelargonium graveolens* L., basil; and *Ocimum basilicum* L. were investigated in laboratory against early fourth instar larvae of the greater wax moth, *G. mellonella*. The tested plant oils

prolonged the larval - pupal periods and reduced both pupation and percentage of adult emergence. *O. basilicum* scored the highest larval mortality followed by *M. piperita* then *P. graveolens* (Elbarky *et al.*, 2015). The plant oils of freed best alternatives to the insecticides for controlling wax moths (Ncibi *et al.*, 2021). Four natural products were examined for GWM control, namely, tobacco extract (*Nicotiana tabacum*), eucalyptus oil (*Eucalyptus* spp.), malagueta pepper (*Capsicum frutescens*) and neem oil (*Azadirachta indica*). The different product concentrations were safe for the bees and effectively controlled the moth where eucalyptus oils and neem at low concentrations caused mortality of wax moth, and they are safe for colony population growth (Telles *et al.*, 2020). The plant oils of freed best alternatives to the insecticides for controlling wax moths (Ncibi *et al.* 2021). Five essential oils (Garlic, *Allium sativum*; camphor, *Cinnamomum camphor*; menthol, *Mentha* sp.; cinnamon, *Cinnamomum verum*; and clove, *Dianthus* sp.) on wax moth larvae and adult honeybee workers under laboratory conditions. The cinnamon, garlic, menthol, clove, and camphor oils were highly effective against wax moth larvae. Moreover, all the essential oils were highly safe for adult honeybee workers under laboratory conditions (Helaly *et al.*, 2022).

Materials and methods

1. Experimental wax moth:

To obtain a pure culture of wax moth, newly emerged wax moth females and males were collected from naturally infested wax combs from the apiary of Plant Protection Research Institute at Quantar, Qalubia Governorate. Materials and the experiment were carried out under the Apiary Conditions boxes and kept in apiary under the weather condition (24 ± 5 °C. and $60 \pm 5\%$ R.H.) till the adult

emergence (Mabrouk *et al.*, 2009) at during the period of 11/5 to 27/7/2016 to study the effect of some natural oils on adult wax moth and on individual activity of honeybee colonies and their comparison with them. Twelve honey bee carniolan were selected. The colonies have been divided into 4 groups (Each of 3 colonies). Colonies in each group were insignificant of infested with adult wax moth.

2. Substances used:

Mint oil 25%, camphor oil 25%, garlic oil and mixture oils.

3. Preparing the natural substances:

Such groups were treated as follows:

Group (A): Mint oil 25% which was prepared on the shape plates from aluminum foil as follow: A mixture of 50g of (Talc powder 30g+Vaseline20g) containing 25% mint oil (12.5 cm³.) and put in aluminum foil inside petri dish as paste form.

Group (B): Camphor 25% which was prepared on the shape plates from aluminum foil as follow: A mixture of 50g of (Talc powder30g+Vaselin20g) containing 25% camphor (12.5 cm³.) and put in aluminum foil inside petri dish as paste form.

Group (C): Garlic oil25% which was prepared on the shape plates from aluminum foil as follow: A mixture of 50g of (Talc powder30g+Vaselin20g) containing 25% garlic oil (12.5 cm³.) and put in aluminum foil inside petri dish as paste form days.

Group (D): Mixture oils (Mint oil, camphor oil and garlic oil) were used by rate, 12.5 cm³ per colony. per week. (3 and at the rate of 1- plate/ 2weeks/colony for 12 weeks at high temperature 27°C. In case of infestation of honeybee colonies with wax mouth, it is advisable to use garlic, camphor and menthol oils.

The plates were placed on the top of board facing the brood chamber after opening the plate cover. The

colonies were treated with (2 plates/colony). The total treatment period was 12 weeks. The dead adult wax mouth was counted and removed at each treatment. Materials are cheap, available to beekeepers, and could be used to control other hive infestations *e.g. Varroa* and acarine mites *.etc.*

4. Effects of tested materials:

Effect of tested materials on honeybee colonies (After treatment of the experimental colonies, the average daily of queen and adult bees were counted until the end of experimental and disturbance of honeybee colonies were observed compared with untreated colonies (Control). on brood rearing activity the daily worker sealed brood cells was counts after treated honeybee colonies at 12 days intervals, on honey production and on pollen production.

5. Statistical analysis:

Obtained data were statistically analyzed; F test and Duncan^s multiple range test were used to compare the means between the group percent mortalities calculated.

Results and discussion

The data regarding mean number of dead adult wax moth recorded a 1,2,3,4,5,6,7,8,9,10,11 and 12 weeks after treatment with mint oil 25%, camphor 25%, garlic oil 25% and mixture 25%. In Table (1) and Figure (1), the data revealed that there were significant differences between treatments. The maximum mean number of dead adult wax moth was 9 in the first week when using camphor 25% while in weeks 2, 3, 4 and 5, the maximum mean number of dead adult wax moth was 7, 8, 8, 9 when using garlic oil 25%. In 6 week, mixture 25% oil was caused the maximum mean number of dead adult wax moth 10. The week 7, 10 and 12 the maximum mean numbers of dead adult wax moth were (9, 9),(9, 8) and (10, 10) when used garlic and camphor 25%oils.The 8 week maximum mean numbers of dead

adult wax moth were 9 when used mixture 25%. The 9 and 11 weeks the maximum mean numbers of died adult wax moth were (8, 8) and (8, 8) when used mint and garlic oils 25%, while the maximum general mean was 8.25

and 7.50 when used garlic and camphor oils 25%, respectively. The minimum general mean number of dead adult wax moth was 6.42 and 6.17 at 12 weeks when used mixture and mint oils 25%, respectively.

Table (1): Mean number of dead adult wax moth after weekly sequence from the treatments.

Treatments	Mean number of dead adult wax moth after treatments /week												
	1	2	3	4	5	6	7	8	9	10	11	12	Mean
Mint oil 25%	5	6	7	6	7	5	6	6	8	7	8	6	6.42
Camphor 25%	9	4	4	7	8	9	9	8	7	8	7	10	7.50
Garlic oil 25%	7	7	8	8	9	9	9	7	8	9	8	10	8.25
Mixture 25%	5	5	2	5	7	10	8	9	5	6	5	7	6.17
F value L .S.D at 5%	6.421 0.415												

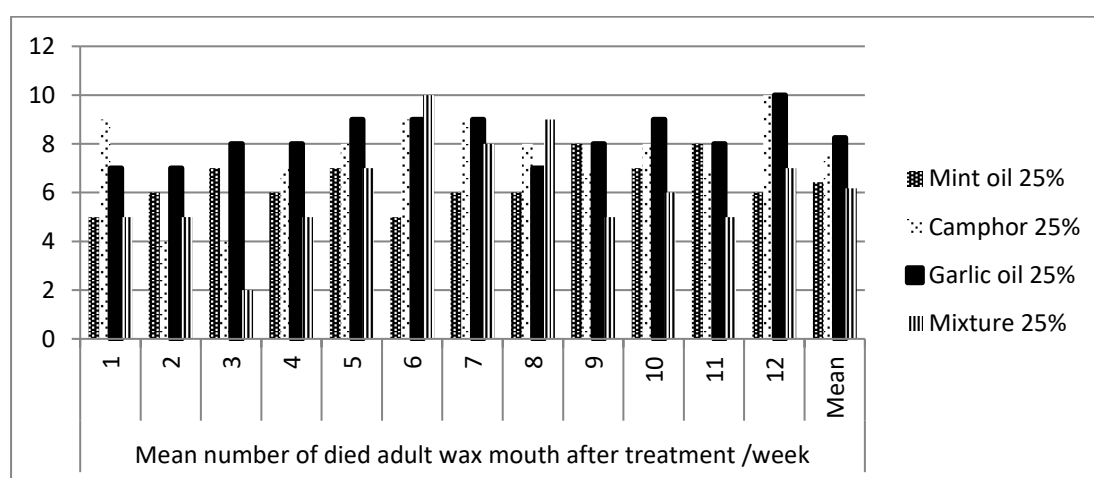


Figure (1): Number of dead adult wax moth after weekly sequence from the treatments.

Table (2) and Figure (2) indicated that the percentage mortality of adult wax moth after weeks sequence from the treatment. The data revealed that natural oils were their effectiveness against adult wax moth. However, a significant difference was present among mint oil 25%, camphor 25%, garlic oil 25%, and mixture 25%. The maximum adult mortality was 100% at

12 weeks when using garlic oil 25% and camphor 25% while the mean was 83% and 75%, respectively. The minimum adult mortality was 70% at 12 weeks when using mixture 25% and 60% when used mint oil 25% while the mean was 62% and 68%, respectively. The data also revealed that all the treatment means differed from one another, which showed that all the natural oils.

Table (2): Percentage mortality of adult wax moth after weekly sequence from the treatment.

Treatment	% mortality												Mean
	1	2	3	4	5	6	7	8	9	10	11	12	
Mint oil	50	60	70	60	70	50	60	60	80	70	80	60	68 ^c
Camphor oil	90	40	40	70	80	90	90	80	70	80	70	100	75 ^b
Garlic oil	70	70	80	80	90	90	90	70	80	90	80	100	83 ^a
Mixture	50	50	20	50	70	100	80	90	50	60	50	70	62 ^d

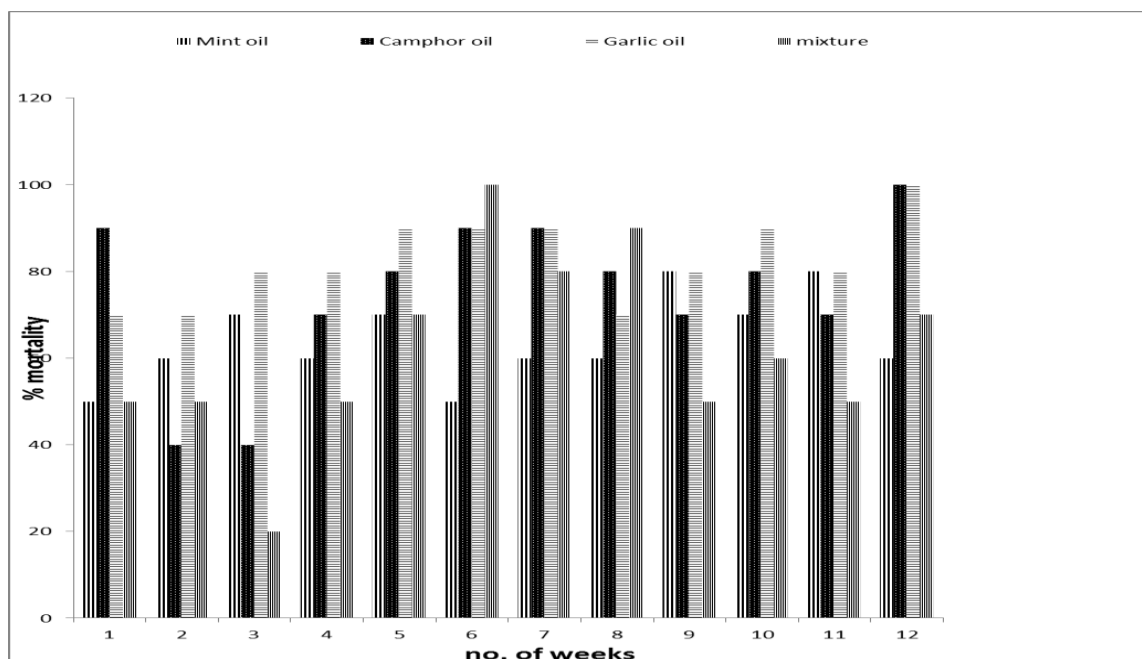


Figure (2): Percentage mortality of adult wax moth after weekly sequence from the treatment.

1. Effect of tested substances odour and individual colony statues:

Table (3) cleared that, there was no effect of tested substances odour and individual colony statues compared with that of untreated colonies.

2. Effect of tested substances on worker sealed brood cells:

As shown in Table (3), the highest average daily worker brood cells were (118.89) cells with an increase of (14.83) when using garlic oil, followed by (95.67) cells with an increase of (14.09) when using camphor oil, (87.15) cells with an increase of (12.07) when using mint oil (71.84) cells with an increase of (12.65) when using a mixture, respectively.

3. Effect of tested substances on honey production:

Table (3) indicated that the highest average of honey production was (375.67) with an increase of (6.40) as compared to that untreated colonies when using garlic oil, followed by (232.00) with an increase of (6.61) when using camphor oil, (226.17 with an increase of (4.30) when using mint oil, (205.50) with an increase of (3.38) as compared to that untreated colonies

when using a mixture for controlling wax moth.

4. Effect of tested substances on pollen production:

Table (3) indicated that the highest average of pollen production was (126.51) with an increase of (18.30) as compared to that untreated colonies when using garlic oil, followed by (102.65) with an increase of (15.14) when using camphor oil, (91.65) with an increase of (13.79) when using mint oil, (72.55) with an increase of (13.69) when using mixture oil as compared to that untreated colonies for controlling wax moth.

It can be concluded that using natural tested substances for controlling adult wax mouth. Result obtained were in agreement with Abdelrahman *et al.* (2012), Calderone (2000), Charrière and Imdorf (1997), Elkhia (2012), Sharaf El-Din (1998) and Swamy *et al.* (2006).

Table (3): Effect of tasted substances on colony status, brood rearing activity, honey and pollen areas.

Treatments	No. of queenless	Average daily of adult bees died after treat.	Average daily of sealed brood cells after treat.	% Increase of sealed brood	Clover honey area	%increase of honey	Pollen area	% Increase of pollen
Mint oil	0.0	3.76	87.15	12.07	221.67	4.30	91.65	13.79
Camphor oil	0.0	3.83	95.67	14.09	232.00	6.61	102.65	15.14
Garlic oil	0.0	4.13	118.89	14.83	375.67	6.40	126.51	18.30
Mixture	0.0	4.45	71.84	12.65	205.50	3.38	72.55	13.69
Untreated	0.0	4.39	62.16	0.53	186.65	2.04	55.22	9.27

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